

the art. This antibody can be labeled with the radioactive isotope  $^{125}\text{I}$ , for example, by methods routinely practiced in the art. In a heterogeneous assay, the GST-GLUTX fusion protein can be anchored to glutathione-agarose beads. The 5 interactive binding partner can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system 10 and allowed to bind to the complexed components. The interaction between GLUTX and the interactive binding partner can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction 15 by the test compound will result in a decrease in measured radioactivity.

Alternatively, the GST-GLUTX fusion protein and the interactive binding partner can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The 20 test compound can be added either during or after the species are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of inhibition of the GLUTX/binding partner interaction can be detected by adding 25 the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of GLUTX and/or the 30 interactive or binding partner (in cases where the binding partner is a protein), in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding

sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the 5 gene encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective proteins will reveal the mutations that correspond to the region of the protein involved in interactive binding. Alternatively, one protein can be 10 anchored to a solid surface using methods described above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain may remain associated with the 15 solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the intracellular binding partner is obtained, short gene segments can be engineered to express peptide fragments of the protein, which can then be tested for binding activity 20 and purified or synthesized.

**XV. Methods for Reducing GLUTX Expression**

Expression of GLUTX can be reduced through the use of modulatory compounds identified through the use of the 25 screening methods described above. In addition, endogenous GLUTX gene expression can also be reduced by inactivating or "knocking out" the GLUTX gene or its promoter using targeted homologous recombination (see, for example, U.S. Patent No. 5,464,764). For example, a mutant, non-functional GLUTX 30 (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous GLUTX gene (either the coding regions or regulatory regions of the GLUTX gene) can be used, with or without a selectable marker and/or a negative

selectable marker, to transfect cells that express GLUTX-3 *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the GLUTX gene. Such approaches are particularly suited for use 5 in developing animal models to study the role of GLUTX; in this instance, modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive GLUTX gene. However, a knock out approach can be adapted for use in humans, provided the recombinant DNA constructs 10 are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous GLUTX gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the GLUTX gene 15 (*i.e.*, the GLUTX promoter and/or enhancers) to form triple helical structures that prevent transcription of the GLUTX gene in target cells in the body (Helene, *Anticancer Drug Res.* 6:569, 1981; Helene *et al.*, *Ann. N.Y. Acad. Sci.* 660:27, 1992; and Maher, *Bioassays* 14:807, 1992).

20 In addition, as discussed above, anti-sense molecules, ribozymes, and peptide nucleic acids can be used to reduce GLUTX expression.

XVI. Assays for the Identification of Compounds that 25 Ameliorate Disorders Associated with Aberrant GLUTX Expression or Activity

Compounds, including, but not limited to, compounds identified via assay techniques such as those described above may be useful for the treatment of disorders 30 associated with aberrant GLUTX expression or aberrant GLUTX activity.

While animal model-based assays are particularly useful for the identification of such therapeutic compounds,